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(54) Title: PROTRACTED GLP-1

(57) Abstract

GLP-1 compounds containing certain protamine and/or metal ions such as zinc have protracted action.

FOR THE PURPOSES OF INFORMATION ONLY

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Protracted GLP-1

FIELD OF THIS INVENTION

The present invention relates to a composition containing GLP-1 compounds and having protracted action and to a process for preparation thereof.

BACKGROUND OF THIS INVENTION

Diabetes is characterized by an impaired glucose metabolism manifesting itself among other things by an elevated blood glucose level in the diabetic patients. Underlying defects lead to a classification of diabetes into two major groups, i.e., type I and type II diabetes. Type I diabetes, also designated insulin demanding diabetes mellitus (IDDM), arises when patients lack ß-cells producing insulin in their pancreatic glands. Type II diabetes, also designated non-insulin dependent diabetes mellitus (NIDDM), occurs in patients with an impaired ß-cell function besides a range of other abnormalities.

Type I diabetic patients are currently treated with insulin while the majority of type II diabetic patients are treated either with agents that stimulate ß-cell function or with agents that enhance the tissue sensitivity of the patients towards insulin.

Glucagon-like peptide-1, also designated GLP-1, is a peptide sequence found as a constituent of mammalian proglucagon. In 1985, it was demonstrated that GLP-1(1-36) amide stimulates insulin release from isolated precultured rat pancreatic islets in the presence of glucose in a dose-dependent manner. This finding suggests that GLP-1(1-36) amide and related peptides might be useful in the treatment of type II diabetes. In recent years, particular interest has focused on GLP-1 fragments such as GLP-1(7-37) and GLP-

1(7-36) amide and analogues and functional derivatives thereof. Hereinafter, these compounds are designated GLP-1 compounds.

It has been found that GLP-1 compounds such as GLP-1(7-37) and GLP-1(7-36) amide have a too fast action when administered to human subjects. Therefore, there is a need for compositions containing GLP-1 compounds and having a protracted action. The availability of such protracted compositions will spare the diabetics the chore and discomfort of multiple daily injections.

Apparently, some theoretical possibilities of controlling the duration of action of GLP-1(7-37) is desribed at the bottom of Page 6 in US patent specification No. 5,120,712. The possibilities mentioned are the use of polymers to complex or adsorb GLP-1(7-37), the selection of appropriate macromolecules (for example, protamine sulphate is mentioned among other), the incorporation of GLP-1(7-37) into particles of a polymeric material or the entrapment of GLP-1(7-37) in microcapsules.

One object of this invention is to provide compositions containing GLP-1 compounds and having a protracted action.

A further object of this invention is to provide compositions containing GLP-1 compounds and having a sufficient high stability.

BRIEF DESCRIPTION OF THIS INVENTION

Surprisingly, it has been found that protracted compositions containing a GLP-1 compound are obtained if said compositions contain protamine and/or a metal salt selected from the group consisting of cobalto and zinc salts.

It is highly surprising that compositions of this invention release the same or almost the same amount of the active compound per time unit during a very long period of time, for example, during a period of a half to one day. In

many cases, such a linear profile of release is the preferred one.

DETAILED DESCRIPTION OF THIS INVENTION

This invention deals with compounds having GLP-1 like activity herein referred to as GLP-1 compounds. GLP-1 compounds bind to the GLP-1 receptor (vide Proc.Nat. Acad.Sci.USA 89 (1992), 8641). Examples of specific GLP-1 compounds are polypeptides comprising the 7 - 33 amino acid sequence of GLP-1, viz. formula I:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val

(I)

or a peptide sequence derived from formula I by exchanging a few of the amino acid residues with other amino acid residues which can be coded for by the nucleotide sequences. Preferably, not more than one, two or three of the amino acid residues have been exchanged. The term GLP-1 compound also comprises derivatives of said polypeptides such as acid addition salts, carboxylate salts, lower alkyl esters, amides, lower alkyl amides and lower dialkyl amides.

As appears from the above statement, the compositions of this invention always contain a GLP-1 compound. In addition to this, said composition contains either protamine or a metal salt selected from the group consisting of cobalto and zinc salts. Alternatively, the compositions of this invention contain both protamine and a metal salt selected from the group consisting of cobalto and zinc salts. If the GLP-1 compound present in the compositions of this invention is GLP-1(7-37) then said composition contains a metal salt selected from the group consisting of cobalto and zinc salts.

The compositions of this invention are prepared in a manner known per se. Being informed about the constituents of the composition, the skilled art worker knows how to prepare such compositions.

For example, the cobalto or zinc salts can be the chloride or another pharmaceutically acceptable salt. Obviously, in the composition of this invention, the cobalto or zinc may be present as free ions and, optionally, a proportion of cobalto or zinc may be bound to other compounds such as the GLP-1 compound and protamine, if present.

Protamine is known to be a mixture of basic polypeptides. Protamine can be obtained from fishes of the family salmon such as Oncorhynchus keta. However, also protamine from other fish can be used. Normally, protamine is marketed as protamine sulphate. However, also other salts can be used. Preferably, protamine of high purity is used.

The compositions of this invention may contain a micelle forming compound in order to stabilize the composition of this invention.

In addition to the specific ingredients which are to be present in the compositions of this invention, said composition usually also contains a liquid diluent, preferably water, and, optionally, a pH buffering agent, an osmotic pressure controlling agent, a preservative or other ancillary agents may be added.

The compositions of this invention can be in any form known by the skilled art worker for such compositions such as a solution or a suspension. A suspension of this invention may contain crystalline and/or amorphous material.

The compositions of this invention may, for example, be used as an insulinotropic agent in the treatment of diabetes. The dosage to be administered to human subjects is conveniently determined by a physician. Normally, the compositions of this invention are administered subcutaneously or intramuscularly.

The features disclosed in the present description, examples and claims may, both separately and in any combination thereof, be material for realizing this invention in diverse forms thereof.

This invention is further illustrated by the following examples which are not to be construed as limiting, but merely as an illustration of some preferred features of this invention. Additional preferred embodiments of this invention are stated in the claims.

Example 1

Trace concentrations of ^{125}I-GLP-1(7-36) amide were added to GLP-1(7-36) amide in the following four preparations designated A, B, C and D:

Preparation A: GLP-1(7-36) amide (1 mg/ml), glycerol (16 mg/ml) and phenol (3 mg/ml); pH value: 7.4.

Preparation B: GLP-1(7-36) amide (1 mg/ml), zinc chloride (0.9 mmol/l), glycerol (16 mg/ml) and phenol (3 mg/ml); pH value: 7.4.

Preparation C: GLP-1(7-36) amide (1 mg/ml), protamine sulphate (500 µg/ml), glycerol (16 mg/ml) and phenol (3 mg/ml); pH value: 7.3.

Preparation D: GLP-1(7-36) amide (1 mg/ml), zinc chloride (0.9 mmol/l), protamine sulphate (500 µg/ml), glycerol (16 mg/ml) and phenol (3 mg/ml); pH value: 7.3.

100 µl of preparation A was injected at one side of the neck and 100 µl of preparation B at the other side in each of 6 pigs. One week later, preparations C and D were injected in the same way in the same pigs. The absorption was followed

by external monitoring of the radioactivity remaining at the site of injection. Residual radioactivity was expressed as percentage of the radioactivity measured at the time of injection. The results obtained appear from table I.

Table I

Time	Percentage Residual Radioactivity			
Hours after injection	A	B	C	D
0	100	100	100	100
1	61	96	-	-
2	31	94	77	93
4	11	80	61	78
6	6	66	51	67
7½	-	-	45	58
23	1	21	-	-
24	-	-	13	15

It appears from this table that the presence of zinc and, optionally, protamine surprisingly results in a prolonged absorption of the GLP-1 compound.

CLAIMS

1. GLP-1 compositions containing, in addition to a GLP-1 compound, protamine and/or a metal salt selected from the group consisting of cobalto and zinc salt, with the proviso that if the GLP-1 compound is GLP-1(7-37) then said composition contains a metal salt selected from the group consisting of cobalto and zinc salts.

2. A composition, according to Claim 1, characterized in that it does not contain protamine.

3. A composition, according to Claim 1, characterized in that it does not contain a cobalto or zinc salt.

4. A composition, according to Claim 1, characterized in that the content of the GLP-1 compound is in the range from about 0.1 through about 50 mg/ml.

5. A composition, according to Claim 1, 2 or 4, characterized in that it contains a cobalto salt.

6. A composition, according to Claim 1, 2 or 4, characterized in that it contains a zinc salt.

7. A composition, according to the previous claim, characterized in that the content of zinc is in the range from about 0.1 through about 10 atoms of zinc per molecule of GLP-1 compound.

8. A composition, according to the previous claim, characterized in that the content of zinc is in the range from about 0.3 through about 5 atoms of zinc per molecule of GLP-1 compound.

9. A composition, according to the previous claim, characterized in that the content of zinc is in the range from about 0.3 through about 1 atom of zinc per molecule og GLP-1 compound.

10. A composition, according to Claim 9, characterized in that the content of zinc is in the range from about 2 through about 4 atoms of zinc per molecule of GLP-1 compound.

11. A composition, according to anyone of the previous claims, characterized in that the GLP-1 compound is GLP-1(7-37).

12. A composition, according to anyone of the Claims 1 through 11, characterized in that the GLP-1 compound is GLP-1(7-36) amide.

13. A composition, according to anyone of the Claims 1 and 3 through 12, characterized in that the composition contains protamine and a metal salt selected from the group consisting of cobalto and zinc salts.

14. A composition, according to the Claims 1 and 3 through 13, characterized in that the content of protamine is so that the molar ratio between protamine and the GLP-1 compound is in the range from about 1:1 through about 1:10.

15. A composition, according to anyone of the previous claims, characterized in that the composition contains a micelle forming compound.

16. A composition, according to the previous claim, characterized in that the micelle forming compound is a phospholipid, preferably lechitin.

17. A composition, according to the previous claim, characterized in that the micelle forming compound is present in a concentration sufficiently high to form micelles.

18. A composition, according to any one of the previous claims, characterized in the pH value thereof is in the range from about 3 through about 8.5.

19. A composition, according to the previous claim, characterized in that the pH value thereof is in the range from about 6 through about 8.

20. A composition, according to anyone of the preceding claims, for use as a pharmaceutical, preferably for subcutaneous or intramuscular administration.

21. The use of a composition described in anyone of the preceding claims as a medicament.

22. A method of treating diabetes which method comprises administering a composition described in anyone of the Claims 1 through 20 in an effective amount to a patient in need of such a treatment.

23. A process for preparing GLP-1 compositions described in any one of the Claims 1 through 20, characterized in that protamine and/or a salt selected from the group consisting of a cobalto and zinc salt is mixed with the GLP-1 compound, optionally in an aqueous medium.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00317

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/26
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, WPI, CA, EMBASE, CLAIMS, JAPIO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A1, 9011296 (THE GENERAL HOSPITAL CORPORATION), 4 October 1990 (04.10.90) -- --------	1,3,4,12,14, 21

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 November 1994	07 -12- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Elisabeth Carlborg Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00317

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 22
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

29/10/94

International application No.
PCT/DK 94/00317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9011296	04/10/90	EP-A- 0464022	08/01/92